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EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF *GINKGO BILOBA* IN RATS

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Abstract : The mechanism of hepatoprotective effects of *Ginkgo biloba (GB)*, an herbal preparation with wide variety of therapeutic application, on paracetamol (Pcml) induced hepatic damage in rats has been ivestigated. *GB* treatment restored the marker enzyme levels indicating the *in vivo* protective effects against Pcml induced liver damage both in preventive and curative aspects. *GB* also rversed the increased TBARS levels, and elevated the GSH content of the liver. The results obtained from the study indicate hepatoprotective nature of *GB*, which might be due to its ability to prevent lipid peroxidation and replenishing the gllutathione level. The effects of *GB* were comparable to that of silymarin.

Key words : *Ginkgo biloba* hepatoprotective paracetamol hepatotoxicity malondialdehyde glutathione rat

INTRODUCTION

Extracts from the leaves of *Ginkgo biloba (GB)* have been used therapeutically since centuries. The effects of *GB* may be caused by a single active ingredient or by the combined action of the many active agents, the most important being flavonoids (Ginkgo-flavone glycosides) and terpenoids (Ginkgolides and Bilobalide) (1). *GB* exhibits a variety of interesting pharmacological activities such as free radical scavenging activity, Platelet - Activating - Factor (PAF) antagonism, cyclic nucleotide phosphodiesterase inhibition, membrane stabilising effect, platelet antiaggregoatory activity, increase in blood fluidity and improvement in cognitive function (2, 3, 4).

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Pcml is a commonly used antipyretic available over the counter. Liver is among the organs most susceptible to the toxic effects of Pcml due to overdosage. One of the mechanisms of hepatotoxicity of a chemical hepatotoxin is the lipid peroxidation medicted by free radicals (5, 6). *GB* is claimed to be an effective antioxidant. Hence the present study was undertaken to evaluate the hepatoprotective effects of *GB* on Pcml induced liver damage and to probe into the mechanism of hepatoprotection. The activity of *GB* was compared with silymarin, a standard hepatoprotective.

METHODS

Drugs and Chemicals: - *Ginkgo biloba* dry extract prepared from the leaves (containing 0.24 mg of ginkgoflavonglycosides/g of dry extract) was a kind gift from Ranbaxy Laboratories, Delhi. Pcml was obtained from Themis Pharmaceuticals. Silymarin pure powder was obtained from Indena s.p.a., Milan, Italy. Thiobarbituric acid (TBA), 5–5'– Dithio-bis-Nitrobenzoic acid (DTNB) and Glutathione (GSH) were obtained from Sigma Chemical Company, St. Louis, MO, USA. All other chemicals were obtained from local sources and were of analytical grade.

Animal: - Adult male albino rats of Wistar strain weighing between 150–250 g bred locally were used in this study. They were housed in clean polypropylene cages and fed with commercial pelleted rat chow (M/S Hindustan Lever Ltd., Mumbai) and water *ad libitum*.

Experimental Procedure: - The experiment was carried out after obtaining clearance from Institutional Animal Ethics Committee. The animals were divided into 7 groups of 6 animals each. *GB* was dissolved in distilled water and given by intraperitoneal route. A suspension of Pcml was prepared in 2% gum acacia and administered orally. Silymarin was also administered in a similar way.

The treatment protocol was planed to study the role of *GB* in both preventive and curative aspects of Pcml-induced hepatotoxicity.

Group I consisted of control rats which received 2% gum acacia orally for 3 days.

Group II received Pcml 2 g/kg body weight orally once daily for 3 days (7).

Group III received Pcml 2 g/kg orally for 3 days followed by 2% gum acacia for the next 7 days. This was done to assess the regeneraive capacity of the liver.

Group IV received Pcml 2 g/kg orally and *GB* 50 mg/kg intraperitoneally (8) simultaneously for 3 days.

Group V received Pcml 2 g/kg orally for 3 days followed by *GB* 50 mg/kg intraperitoneally for next 7 days.

Group VI received Pcml 2 g/kg orally and silymarin 200 mg/kg orally (9) simultaneously for 3 days.

Group VII received Pcml 2 g/kg orally for 3 days and then silymarin 200 mg/kg orally for 7 days.

At the end of treatment period, blood samples were collected by direct cardiac puncture and the serum separated for different biochemical analyses. The rats were sacrified by cervical dislocation. The livers were immediately excised, washed in ice-cold normal saline and blotted dry with filter paper. A 50% (w/v) homogenate was prepared using 0.05M sodium phosphate buffer (pH 7.0). The homogenate was centrifuged at 700 xg for 10 minutes at 4°C in a refrigerated centrifuge and the supernatant was used for the estimation of lipid peroxidation and glutathione.

Enzyme Assays: - The activities of serum hepatic marker enzymes namely aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were assayed in serum using standard kits from Lupin Laboratories and Pointe Scientific respectively. The results were expressed as units/litre (IU/L).

Protein Estimation:-

The levels of proteins i.e., total proteins (TP) and albumin (Alb) were estimated in serum of experimental animals by biuret method and bromcresol green method respectively. Standard kits from Ranbaxy Laboratories, Delhi were used for these estimations.

Lipid Peroxidation: - The quantitative measurement of lipid peroxidation was done by measuring the concentration of TBARS in liver using the method of Ohkawa (10). The amount of malondialdehyde (MDA) formed was quantitated by reaction with thiobarbituric acid (TBA) and used as an index of lipid peroxidation. The results were expressed as nanomole of MDA released/g of wet tissue using molar extinction coefficient of the chromophore (1.56×10^{-5} M^{-1} cm^{-1}).

Glutathione Estimation: - GSH was estimated in the liver homogenate using DTNB by the method of Buetler (11). The absorbance was read at 412 nm and the results were expressed as mg of GSH/g of wet tissue.

Histopathologic Examination: - Small portions from the right and left lobes of liver were quickly dissected out from the animals after autopsy and processed for routine microtomy. Sections were made about 4–6 µm in thickness. They were stained with hematoxylin and eosin (H&E) and photographed.

Statistical Analysis: - The statistical analysis was carried out by One-way Analysis of Variance (ANOVA) followed by studentized range procedure.

RESULTS

In the present study, a dose of 2 g/kg of Pcml was used to induce liver damage in the rat as indicated by a significant ($P<0.05$) elevation of serum marker enzymes namely

AST, ALT and ALP and a significant ($P<0.05$) reduction of serum TP and Alb (Table I). These results corroborate with the earlier reports (12, 13, 14). Histopathological changes seen confirmed hepatic damage. Compared to the normal liver tissue (Fig. 1), Pcml treatment showed extensive centrilobular necrosis extending to midzone with neutrophilic collection. Central to central bridging necrosis was seen. There was mild chronic inflammatory cell infiltrate in the portal tracts. In some of the lobules focal centrizonal necrosis was seen. In few lobules the hepatocytes around the central vein showed apoptosis i.e., intense eosinophilia of hepatocytes with pyknotic nuclei (Fig. 2).

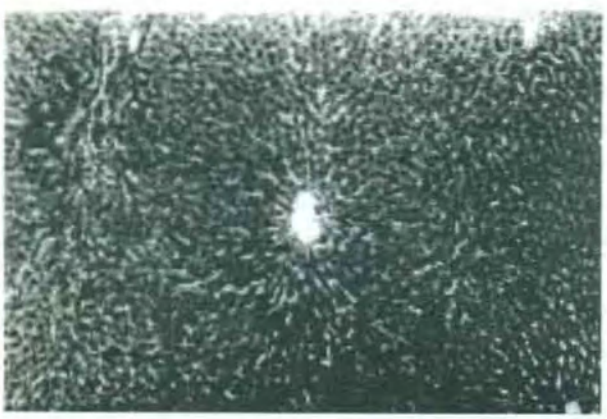

Fig. 1: T.S. of liver tissue showing normal histology.

Fig. 2: T.S. of liver tissue of Pcml treated rats showing extensive centrilobular, midzonal and central-to-central bridging necrosis. Only periportal hepatocytes are viable.

TABLE I: Effect of *Ginkgo biloba (GB)* and Silymarin on serum marker enzymes and serum proteins in paracetamol (Pcml) induced liver damage

Group	*Drugs*	*AST (IU/L)*	*ALT (IU/L)*	*ALP (IU/L)*	*TP (g/dl)*	*Alb (g/dl)*
I	2% Gum acacia (Control)	66.83±4.02	31.17±2.09	445.33±23.16	8.15±0.26	6.37±0.25
II	Pcml (Hepatic Injury)	348.17±37.40[a]	256.33±25.30[a]	956.83±49.40[a]	4.93±0.11[a]	2.22±0.21[a]
III	Pcml followed by 2% Gum acacia (Regeneration aassessment)	302.33±34.10[a]	212.67±19.12[a]	791.17±44.31[a]	5.20±0.36[a]	3.20±0.28[a]
IV	Pcml and GB (Preventive)	77.83±8.93[b]	42.50±6.60[b]	423.17±77.27[b]	9.31±0.74[b]	6.03±0.59[b]
V	Pcml followed by GB (Curative)	82.33±5.90[b]	44.50±6.10[b]	536.17±60.76[b]	8.36±0.49[b]	6.71±0.53[b]
VI	Pcml and silymarin (Preventive)	70.33±4.85[b]	33.17±2.27[b]	418.67±26.33[b]	9.77±0.36[b]	6.51±0.58[b]
VII	Pcml followed by silymarin (Curative)	61.17±5.71[b]	37.17±3.96[b]	505.83±71.50[b]	10.22±0.52[b]	7.73±0.66[b]

Values expressed as Mean ± SEM
Number of animals in each groups = 6
a $P<0.05$ Vs Group I b $P<0.05$ Vs Groups II & III

Both preventive and curative aspects of hepatoprotection were studied. Liver has got capacity to regenerate. This regenerative capacity was assessed by administering Pcml 2 g/kg orally for 3 days and then with vehicle for 7 days (Group III). The results as shown in Table II indicate that no regeneration has taken place. This was further supported by the histopathological examination which still showed necrotic changes. This group also acted as a control for curative treatment. The leaf extract of *GB* exhibited an ability to counteract the Pcml induced changes in the biochemical parameters both in preventive and curative aspects. This was supported by the histopathologic study wherein co-administration of either *GB* (Fig. 3) or silymarin (Fig. 5) improved the histological picture of liver. In the curative groups of both *GB* (Fig. 4) and silymarin (Fig. 6), evidence of residual hepatocellular necrosis with cords of regenerating hepatocytes were seen.

Fig. 3: T.S. of liver after simultaneous treatment of *GB* and Pcml (preventive) showing no evidence of liver cell damage.

Fig. 4: T.S. of liver after treatment with Pcml followed by *GB* (curative) showing residual hepatocellular necrosis with cords of regenerating hepatocytes.

Fig. 5: T.S. of liver after simultaneous treatment of silymarin and Pcml (preventive) showing no evidence of liver cell damage.

In an attempt to understand the mechanism by which *GB* prevents hepatic damage caused by Pcml, investigations on levels of TBARS and glutathione were carried out both in preventive and curative groups. TBARS levels were found to be elevated after the administration of Pcml which was significantly ($P<0.05$) reversed

TABLE II: Effect of *Ginkgo biloba (GB)* and Silymarin on thiobarbituric acid reactive substances (TBARS) and glutathione (GSH) levels in liver homogenate in paracetamol-induced liver damage

Group	*Drugs*	*TBARS (nM of MDA/g of wet tissue)*	*GSH (mg/g of wet tissue)*
I	2% Gum acacia (control)	3.16±0.19	1.90±0.09
II	Pcml (Hepatic injury)	3.42±0.30	1.56±0.16
III	Pcml followed by 2% Gum acacia (Regeneration aasessment	3.35±0.17	1.61±0.19
IV	Pcml and GB (Preventive)	2.08±0.13[a]	2.36±0.19[a]
V	Pcml followed by GB (Curative)	2.36±0.18[a]	2.67±0.28[a]
VI	Pcml and Silymarin (Preventive)	1.96±0.13[a]	2.74±0.20[a]
VII	Pcml followed by Silymarin (Curative)	2.10±0.20[a]	2.98±0.14[a]

Values expressed as Mean ± SEM
Number of animals in each groups = 6
MDA = Malondialdehyde
GSH = Glutathione
a. $P<0.05$ Vs Groups II & III

by *GB*. There was a rise in GSH content of liver after treatment with *GB* (Table II). The activity of *GB* was compared with that of silymarin, a proven hepatoprotective. Silymarin also showed reduced serum marker enzyme levels, increased serum proteins. The effects *GB* were comparable to silymarin both in preventive and curative aspects.

Fig. 6: T.S. of liver after treatment with Pcml followed by silymarin (curative) showing residual hepatocellular necrosis with cords of regenerating hepatocytes.

DISCUSSION

Pcml is mainly metabolized by glucuronidation and sulfate conjugation (16). A small amount of Pcml is metabolised by cytochrome P–450 enzymes to a toxic metabolite. The hepatic cells are normally protected from injury by conjugation of this toxic metabolite with glutathione content of hepatocytes available for detoxification. When glutathione gets exhausted, the hepatocytes become vulnerable to the noxious effects of the metabolite resulting in necrosis of the liver (5). *GB* was able to prevent hepatic injury in preventive groups and enhance regeneration in curative groups. This was evidenced by lowering of serum marker enzymes, absence of cell necrosis and almost normal histopathological appearance.

Hepatocellular necrosis leads to elevation of the serum marker enzymes which are released form the liver into blood.

Elevation of these enzymes in serum indicates membrane damage. ALT is a better index of liver injury, as liver ALT activity represents 90% of the total enzyme present in the body (15). More than 50% decrease in these enzymes indicates recovery of the hepatocytes in spite of insult due to hepatotoxin e.g., Pcml.

Hepatotoxicity may be due to lipid peroxidation, depletion of glutathione/ cytochrome P–450, an altered immunological system, induced by various chemical agents or direct damage to the cell (5, 6, 17). Irrespective of the mechanisms of injury, it is clear that ultimate hepatic necrosis is brought about by increased lipid peroxidation or depletion of glutathione. It has been reported that both *GB* and silymarin are antioxidants (18, 19, 20, 21, 22, 23). In out study, similar observations were seen with both *GB* and silymarin which enhanced hepatic glutathione level and inhibited lipid peroxidation. This may probably be one of the mechanisms of hepatoprotective activity of *GB*.

The results of our study indicate that *GB* protects the liver against Pcml induced hepatotoxicity which may be mediated through its antioxidant activity.

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